# Lay summary

We found that female field crickets, which use male acoustic signals to locate mates, were unable to do so when exposed to human generated noise. The amplitude (volume) of the noise was shown to not be causing this effect alone. As similar acoustic behaviors are used by a variety of animals, and as human generated acoustic pollution is becoming more prevalent, these findings have important implications for mate location systems throughout the animal kingdom.

# Anthropogenic noise disrupts mate searching in *Gryllus bimaculatus*

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Running Title: Mate Searching under Anthropogenic Noise

## Abstract

Many animals use acoustic communication as a means of sending important biological information, such as their location, to potential receivers. However, anthropogenic noise is known to affect the ability of some species to either produce or receive signals, which may influence their reproductive success. In this study, we investigate the effect of anthropogenic noise on the mate searching behaviors of the field cricket *Gryllus bimaculatus*. To accomplish this, phonotaxis trials were conducted with female field crickets under different acoustic conditions, and their ability to detect and move towards conspecific male calls was assessed. The presence of traffic noise reduced the likelihood that the female would approach the male calls and also reduced the time that the female spent attending to the calling stimulus before making her decision. However, the presence of white noise did not reduce the likelihood of approaching the calling speaker, indicating that the average amplitude of anthropogenic noise is, alone, not important in this conflict, but frequency and fluctuations in the stimulus or other characteristic might be. This study supports the hypothesis that anthropogenic noise does indeed influence the detectability of acoustic mate location signals, thus disrupting mate searching behavior.



*Key words*: anthropogenic noise, acoustic signals, mate searching, *Gryllus bimaculatus*, sexual signals

## Introduction

Many animal communication systems are heavily influenced by sexual selection due to their importance to reproductive success (Andersson 1994). Of these systems, some fall into the broad category of 'mate location signals' which are signals that have evolved, at least in part, to convey information about the location of the signaller so that a conspecific receiver can locate them and mating can occur (Tyack 1981; Rutowski et al. 2001; Shine et al. 2005; Allen et al. 2012). Species that utilize these signals include those that are solitary or dispersed over large home ranges (e.g. male Empress Leilia butterflies, *Asterocampa leilia*, detect females using visual signals; Rutowski et al. 2001), but these signals are also observed in species which live socially, or in close proximity to conspecifics (e.g. female garter snakes, *Thamnophis* spp., signal to attract males using pheromones; Shine et al. 2005). Thus, mate location occurs through different social structures, but also through different modalities. Perhaps the most well-known modality associated with mate location is acoustic communication, due to its occurrence throughout vertebrate (Naguib et al. 2009) and invertebrate taxa (Pollack 2010). A reduction in the ability to transmit or receive these signals could be detrimental to an individual's reproductive success and overall population stability (Griesinger et al. 2011).

One mechanism that may specifically interfere with the ability of animals to transmit acoustic information is the globally increasing occurrence of acoustic pollution (hereafter referred to as anthropogenic noise). This pollutant is present in both aquatic (McDonald et al. 2006) and terrestrial biomes (Watts et al. 2007), with its potential impacts being partially due to the abundance and diversity of it sources, such as transport networks (Lee et al. 2014), development projects (Lee et al. 2015), and high densities of people (Ammon et al. 2015). Furthermore, many of these sources produce relatively low frequency acoustics (Waye and Rylander 2001), which suffer less attenuation (Berglund et al. 1996) and can travel further

from their source. These characteristics, coupled with increasing development and urbanisation (United Nations 2015), suggest that anthropogenic noise has the strong potential to be a threat to nearby environments and their inhabitants by conflicting with their acoustic signals.

The evolution and plasticity of acoustic behaviors allows species to exploit particular acoustic niches and adapt to environmental changes in order to maximize signal transmission. For example, many acoustic signals that are subject to high levels of background noise are adapted to cope with this competition (Wollerman and Wiley 2002). However, anthropogenic noise may still conflict with these signals, leading to the alterations in signalling behaviors observed in a wide range of taxa, such as birds (Luther et al. 2015), amphibians (Cunnington and Fahrig 2010), fish (Holt and Johnston 2014), mammals (Melcon et al. 2012), and invertebrates (Orci et al. 2016). These alterations often involve pitch modulation to avoid conflicting with similar frequency bands of anthropogenic noise (Cunnington and Fahrig 2010; Luther et al. 2015). Other behavioral alterations include shifts to different signal modalities (Partan 2017), increases in amplitude to decrease signal to noise ratio (Cunnington and Fahrig 2010; Holt and Johnstone 2014; Luther et al. 2015) as well as changes to the timing (Melcon et al. 2012: Orci et al. 2016) and spatial use of signalling (Cunnington and Fahrig 2010) to avoid competition with anthropogenic noise. These alteration are constrained by the plasticity of the species or signalling system in question, so signalling individuals may not be able to alter aspects of their acoustic behavior to avoid conflicting with anthropogenic noise. For example, many avian species can change the frequency of their song (e.g. *Zonotrichia leucophrys nuttalli*; Luther et al. 2015) via behavioural plasticity due to their muscular vocal organs and ability to sing at multiple frequencies, whereas mature crickets (e.g. *Gryllus bimaculatus*) would be unable to do this due to the fixed structures used for stridulation (Bennet-Clark

84 1989), although there is now emerging evidence of long-term changes occurring in other
85 Orthoptera (Lampe et al. 2014).

86 Due to the necessity of mate location signals for the discovery of potential mates,
87 alterations made to these signals to compensate for the presence of anthropogenic noise are
88 strongly associated with ameliorating potential costs to an individual's fitness. However, a
89 recent review (Read et al. 2013) highlights the extent to which studies on anthropogenic noise
90 specifically fail to address the associated costs to the reproductive success of signallers and
91 receivers, whilst instead concentrating on the 'benefits' of apparently adaptive responses,
92 even though empirical evidence of these benefits is scarce (Slabbekorn 2013). Studies
93 exploring the sexual consequences of anthropogenic noise are now becoming more familiar
94 (Schmidt et al. 2014, Gurule-Small and Tinghitella 2018), but these mostly fail to address the
95 mechanisms behind these consequences, demanding further investigation in this area.

96 The aim of this study was to investigate the conflict between acoustic mate location
97 communication and low frequency anthropogenic noise. Specifically, we examined the ability
98 of individuals to detect conspecific sexual signals under noise conditions, and the mechanisms
99 behind this conflict, to quantify the potential costs of signalling under these conditions (Read
100 et al. 2013). As there exists a gap in knowledge when discussing the impacts of anthropogenic
101 noise on invertebrates (Morley et al. 2014), the Mediterranean field cricket, *Gryllus*
102 *bimaculatus*, was chosen as a suitable model system to address our question. The males of this
103 species produce a long range signal, known as a "calling song", to attract potential mates to
104 their location (Alexander 1961). Conspecific females perceive this signal using specialized
105 organs on their front tibia, known as the tympanum, and navigate their way towards the signal
106 via phonotaxis (Hirtenlehner and Römer 2014). Furthermore, the auditory tuning of female
107 *G.bimaculatus* is well understood (Popov et al. 1978), meaning a more comprehensive

conclusion on the different aspects of anthropogenic noise can be inferred. In this experiment, females were exposed to three acoustic conditions with distinct characteristics (ambient, traffic or white noise) and observed in their ability to locate the calling songs of a group of males. If anthropogenic noise conflicts with acoustic signals due to average amplitude alone, then we would expect to see compromised phonotaxis under both traffic noise and white noise conditions. However, if the critical component causing interference is another characteristic, such as the frequency or signal fluctuations of this stimulus, or a combination of factors, then we would expect to see compromised phonotaxis only under traffic noise conditions.

## Materials and Methods

*Study Organisms*

Using individuals sourced from an entomological supplier (Cambridge Reptiles, Cambridge, U.K.), a breeding colony of *Gryllus bimaculatus* was established in a temperature controlled laboratory maintained at 26$^{o}$C, on a 12:12 hour light:dark cycle (simulating 06:00 sunrise and 18:00 sunset). The room was also kept at ambient noise levels, not including the sounds produced by the crickets (44.3±3.8 dBA, measured through a CEM DT-8852 IEC 61672-1 class 2 compliant handheld sound level meter over a period of 1 hour). Juvenile females were separated from the males prior to eclosion to ensure that only virgins were used in behavioral trials, as mating status can alter phonotaxis response behavior in *Gryllus* spp. (Lickman et al. 1998). All individuals received a diet of fresh vegetables, rabbit pellets and water *ad libitum*. Only adult virgin females, free from visible damage, of three weeks post eclosion or younger were observed in this experiment, as they become less receptive after this time (Adamo and Hoy 1994). Prior to acoustic trials, females were weighed (in grams) and measured by taking

the width of their pronotum (in mm). Females were only used in one trial each to avoid a reduction in stimuli response or habituation to acoustic conditions.

*Acoustic Stimuli*

The three main acoustic stimuli created for this study were *Gryllus bimaculatus* calling song, road traffic noise, and generated white noise (see electronic supplementary material S1), and the ambient noise conditions of the room were also utilized (44.3±3.8 dBA). For the calling song, a group of mature virgin males (approximately 20 individuals, varying in quality indicators such as age and size to negate any effect on quality perception or mate preference shown by the female) were recorded using a RØDE NTG4+ shotgun microphone connected to a TASCAM DR-07 MKII linear PCM recorder (.wav format, 16-bit resolution and 48 kHz sampling rate). The microphone was set up adjacent, at a distance of 15cm, to a 30 x 17 x 20 cm plastic tank that held the individuals being recorded. To encourage naturalistic singing behavior, the tank was also equipped with crumpled newspaper to create a 3-D environment with burrows, from which males naturally sing (Simmons 1988). A 30 minute long recording was obtained at a time between 11:00-13:00 and between 24°C-28°C to match the conditions used in the phonotaxis trials. Simultaneously, the Sound Pressure Level (SPL) of the calling individuals was also measured using a CEM DT-8852 IEC 61672-1 class 2 compliant handheld sound level meter (56.2±6.1 dBA). The recording was edited using Audacity 2.1.2 (http://www.audacityteam.org/) to remove extended periods of silence (2+ seconds) and excessive periods of courtship singing, resulting in a 20 minute "Song" stimulus (see electronic supplementary material S2).

Two novel acoustic stimuli, traffic noise and white noise, were used to measure the effects of different noise characteristics on female behavior. Traffic noise is a suitable

representation of anthropogenic noise as it is low in frequency (approximately 0.5-6 kHz), has fluctuating characteristics (such as amplitude), and is common in urban areas (Lee et al. 2014). For this experiment, the recording was conducted using the same equipment as the calling song recording. A 30 minute recording was staged during rush hour (16:00-18:00) and at a five meter distance from the A14 road (South Cambridgeshire) with SPL being simultaneously measured using the handheld sound level meter (79.1±3.5 dBA). Audacity was used to edit out excessive periods of quiet (5+ seconds), resulting in a 20 minute long "Traffic noise" stimulus (see electronic supplementary material S3). White noise, on the other hand, is an electronically generated stimulus that covers all frequency bands at the same level of intensity. For this experiment, a 20 minute "White noise" stimulus, at the same average amplitude as the "Traffic noise" stimulus (79.1 dBA), was generated using Audacity (see electronic supplementary material S4). The relative acoustic power (amplitude) of both of these stimuli were calculated using output from the *ama()* function (50 kHz sampling frequency, absolute amplitude envelope) from the package seewave (Sueur et al. 2008) in Rstudio (Rstudio Team 2016; R Core Team 2017). At 4.5-5.5 kHz (the frequency band which is most sensitive to female *G.bimaculatus*; Popov et al. 1978), the traffic and white noise stimuli had similar relative power (2.7% and 2.2% respectively).

*Phonotaxis trials*

Phonotaxis trials were conducted in a 70cm x 49cm behavioral arena, constructed from corrugated plastic, surrounded by a 10cm high wall with a speaker embedded into each end wall (see electronic supplementary material S5). Playback stimuli from these speakers were checked to be the same average amplitude (from the center circle) as the initial recordings by measuring SPL through the same handheld sound level meter as before. The

180 inside of the arena was visibly split into several areas, including a central circle (diameter=6cm,
181 the same as the cup used to release the females into the arena) and two half circles by each
182 speaker (radius=8cm, >1% of the overall area of the arena) to designate an area where
183 individuals had made a "choice". A video camera (SONY HDR-CX625 HANDYCAM®) was
184 secured above the arena to record each observation. All observations conducted in the arena
185 took place between 11:00-13:00 to minimize time of day affecting phonotaxis behavior, as it
186 does in other *Gryllus* behaviors (Zuk 1987) and at 24-28$^{o}$C.

187 Thirty replicate trials were conducted for each acoustic condition, resulting in a total of
188 90 trials (ambient noise *n* = 30, traffic noise *n* = 30, white noise *n* = 30). For each trial, a
189 randomly selected virgin adult female was placed under a transparent plastic cup (diameter =
190 6 cm) on the central circle and left to acclimatize for five minutes, under ambient noise
191 conditions. After this acclimatisation period, the "Song" stimulus was played from one of the
192 speakers (switched pseudo-randomly between trials so that there were the same number of
193 trials involving calls being produced from the left and right speaker). The acoustic condition
194 (traffic noise or white noise) was broadcast from both speakers, or not at all for ambient noise
195 conditions, so choice was not linked to condition preference. The cup was removed as soon as
196 the song started, allowing the female to move freely around the arena. Trials continued for a
197 period of ten minutes, or until the individual made a 'choice' (entered the half circle next to
198 either speaker: see electronic supplementary material S5). The individual was then removed
199 and the arena was cleaned with 70% ethanol and wiped dry to remove any olfactory cues
200 ahead of subsequent trials. Control trials were also conducted and interspersed with the main
201 trials to measure possible spatial biases within the arena (i.e. side preference), and compare
202 behavioral results between standard and control trials. These were trials that featured the
203 same setup as the standard trials, but no calling song was broadcast, so females reacted to the

non-directional acoustic condition alone. Thirty replicate control trials were conducted, leading to a total of 90 control trials (ambient noise control *n* = 30, traffic noise control *n* = 30, white noise control *n* = 30). Footage of each trial was event coded through the use of BORIS (Behavioral Observation Research Interactive Software; Friard and Gamba 2016). For each trial, the female 'choice' was recorded (an individual made a 'correct' choice if she entered the half circle next to the calling speaker, and an 'incorrect' choice if she entered the half circle next to the non-calling speaker). In addition, an individual's latency to move (leave the starting area) and latency reach a speaker (time taken from leaving starting area to making a 'choice') were also measured.

*Statistical methods*

To prevent outliers from skewing the analysis, statistical outliers were detected and removed from the dataset (ambient noise *n*=1, traffic noise *n*=2, white noise *n*=0, ambient noise control *n*=2, traffic noise control *n*=2, white noise control *n*=4) using a generalized extreme Studentized deviate (GESD) test to the level of *k*=5 for each acoustic condition. This did not change the outcome of primary analysis, but did allow for clearer post-hoc analysis. A generalized linear model (GZLM) using a binary logistic function was used to test for difference in choices between acoustic conditions, and was also coupled with a Tukey HSD multiple comparison analysis. Presented effect sizes (Log odds ratio ± SE) were acquired from the model summary. One-way Chi-Squared tests were used to determine if choice ratios were different to a random distribution in each acoustic condition. All scale data used in analysis was tested for normality using a Shapiro-Wilk tests, and suitable non-parametric analysis were conducted when necessary. A Kruskal-Wallis one-way ANOVA was used to check differences in weight and size between acoustic conditions. Differences between the two different measures

of latency between acoustic conditions were analyzed using a Kruskal-Wallis one-way ANOVA. These were coupled with Dunn test post hoc tests with bonferroni corrections to identify between-group differences. Arena side biases were checked using Chi-squared tests, and the effect of the calling song, female pronotum width (mm), female mass (g), and ambient temperature ($^{o}$C) on latency was checked using Spearman's rank correlation tests and Wilcoxon signed-rank tests. All statistical analysis was conducted with the statistical package RStudio 1.0.134 (Rstudio Team 2016; R Core Team 2017) with the packages 'dunn.test' (Dino 2017), 'Hmisc' (Harrell 2017) and 'multcomp' (Hothorn et al. 2008).

## Results

*Size and weight differences*

There was no difference between the size (Kruskal-Wallis: $X^2_2$=0.171 $n_1$=29, $n_2$=28, $n_3$=30, P=0.918) or weight (Kruskal-Wallis: $X^2_2$=5.114 $n_1$=29, $n_2$=28, $n_3$=30, P=0.078) of individuals between acoustic conditions. There was also no difference between the size (Kruskal-Wallis: $X^2_2$=3.929 $n_1$=28, $n_2$=28, $n_3$=26, P=.0140) or weight (Kruskal-Wallis: $X^2_2$=3.175 $n_1$=28, $n_2$=28, $n_3$=26, P=0.204) of the individuals between acoustic conditions in the control trials.

*Mate location choices*

Females did not show any side preference within any of the acoustic control conditions (Ambient Noise: $X^2_1$=1.286, n=28, P=0.257; Traffic Noise: $X^2_1$=0.571, n=28, P=0.449; White Noise: $X^2_1$=0.154, n=26, P=0.695), or any difference between acoustic conditions ($X^2_2$=0.26047, N=82, P=0.878). Choices made by females in standard trials were influenced by the acoustic

condition presented (GLZM {b}: Likelihood $X^2_2$= 7.462, N=86, p=0.024). Fewer individuals approached the calling speaker in both traffic noise (-1.57±0.49) and white noise trials (-1.16±0.62) compared to those under ambient noise conditions (Figure 1). Females under traffic noise conditions made almost three times as many 'incorrect' choices as those under ambient conditions (n=57, z=-2.530, p=0.031). By proportion, choices made under traffic noise conditions match a random distribution of choices ($X^2_1$=0, n=28, p=1). There was no significant difference in female choice between ambient and white noise conditions (n=58, z=-1.886, p= 0.142), or between traffic and white noise conditions (n=59, z=0.764 p=0.724).

*Choice latency*

Individuals under traffic noise conditions chose more quickly than those in ambient and white noise conditions (Table 1, Figure 2). The driving factor here was the latency to leave the starting area, not the latency to reach a speaker. Individuals under traffic noise conditions left the starting area more quickly than individuals in either ambient or white noise conditions (Figure 2), but there was no significant difference between the latency to reach a speaker between the acoustic conditions. A similar, but non-significant, trend is seen in the control trials with a potential difference between latency to move, but this was not significant (Table 1).

## Discussion

Our results demonstrate that anthropogenic noise can affect the ability of mate-searching individuals to locate potential mates via acoustic signalling behaviors. Furthermore, this acoustic interference is not driven simply by the average amplitude of the noise in the environment, but is potentially due to the low frequency or fluctuating characteristics of the

stimulus, or by a combination of these factors. Traffic noise significantly affected the mate-locating behavior of female *Gryllus bimaculatus*, both in terms of finding calling males and in their latency to do so. These results support those of a previous study that show reduced *Gryllus* phonotaxis capabilities under anthropogenic noise conditions (Schmidt et al. 2014), whilst revealing more about the acoustic mechanisms driving the changes, which may have profound implications for an individual's fitness.

There was a clear difference in the number of 'correct' choices made between the three acoustic conditions. Females did not differentiate between the calling and non-calling speakers under traffic noise conditions, and the behavior of females in the white noise trials was intermediate between traffic noise and ambient noise conditions. These observations thus provide evidence in support of the hypothesis that anthropogenic noise is conflicting with these signals due to its frequency characteristics. Both *Gryllus bimaculatus* calls and anthropogenic noise have relatively low frequencies (<5 kHz, approximately 4-5 kHz and 0.5-6 kHz respectively, see supplementary material S1). This phenomenon is known as masking, and occurs when irrelevant noise decreases the likelihood of detecting or recognising the desired signal (reviewed by Naguib 2013). By altering the frequency components of an acoustic signal, individuals can avoid the effects of masking from anthropogenic noise, a behavior that has been observed in anurans (Cunnington and Fahrig 2010), insects (Lampe et al. 2012), and birds (McMullen et al. 2014; Potvin et al. 2014), providing further evidence that the conflict occurs due to sharing similar frequencies. However, some animals adopt alternative strategies for competing with masking background noise, including increased signalling effort (Cunnington and Fahrig 2010; Holt and Johnston 2014), which would suggest that frequency is not the only factor causing this acoustic conflict.

296 Evidence for another characteristic (such as modulations/fluctuations of a stimulus)
297 being the driving factor is also present in these results. Due to the narrow auditory tuning of
298 *G.bimaculatus* (Popov et al. 1978), females are much more sensitive to frequency bands
299 around 4.5-5.5 kHz. Interestingly, both stimuli used in this experiment had a similar relative
300 acoustic power at this frequency band (2.7% and 2.2% respectively). As these stimuli were
301 played at the same average amplitude, then the power at this frequency band would have also
302 been similar, suggesting that the amplitude and frequency of the noise may not be the main
303 factor of this acoustic conflict. An alternative way that these two stimuli differ is in the
304 fluctuations within the signal. The traffic noise stimulus has fluctuations in both its amplitude
305 and frequency distribution, which is a common characteristic also seen in other forms of
306 anthropogenic noise. However, the white noise stimulus is constant in frequency spectrum and
307 amplitude, meaning it differs from anthropogenic noise notably in this regard. Through
308 exposure to this predictable, non-random noise, individuals may be able to habituate or
309 increase their tolerance to such noises (Nedelec et al. 2015; Kern and Radford 2016). Thus, the
310 amount and intensity of fluctuations in an acoustic stimulus could be a key factor in the
311 conflict with acoustic signals.

312 A further acoustic effect that may compromise communication systems is that of
313 'distractions', which use up an individual's finite attentional capabilities (Naquib 2013). This is
314 mostly dependent on cognition and the neural processing of different sounds (i.e. many
315 different frequencies), and fluctuating noises, such as the passing of vehicles, are less
316 detrimental to attentional capabilities than constant noises (Vélez and Bee 2011). As such, it is
317 unlikely that this is the effect seen with traffic noise in this study, as it has a small dominant
318 frequency range (0.5 – 6 kHz) and fluctuations in amplitude (79.1 ± 3.5 dBA) and other acoustic
319 characteristics (Lee et al. 2014). However, the distraction effect could be involved in the white
320 noise trials, as this does broadcast at multiple frequency bands and is a constant in its

321 characteristics (i.e. frequency, amplitude etc.). However, as these hypotheses are not mutually
322 exclusive, it is plausible that the conflict between anthropogenic noise and acoustic signals is
323 due to a mixture of factors, such as frequency, amplitude, and the modulation of the acoustic
324 characteristics.

325 Interestingly, studies in *Oecanthus* tree crickets have shown that females suffer no
326 reduction in their phonotaxis abilities (Costello and Symes 2014), making them an intriguing
327 comparison to the *Gryllus* species used in this study. Potentially, the difference in outcomes
328 between these studies could be due to the active auditory tuning present in *Oecanthus* species
329 (Mhatre et al. 2016). This ability to alter their hearing under different environmental
330 conditions, for which there is no evidence in *Gryllus* species, would offer a strong selective
331 advantage in rapidly changing environments, such as those affected anthropogenic
332 disturbance.

333 Anthropogenic noise not only influenced a female's ability to approach a calling male,
334 but also the time taken to do so. Females under traffic noise conditions were much quicker to
335 initiate movement and thus approach a speaker sooner than those in either white or ambient
336 noise conditions. However, there was no difference in the time spent actually in motion
337 between the acoustic conditions, meaning that females did not move quicker but actually
338 spent less time attending the acoustic stimuli present. This reduction in latencies can, in turn,
339 affect an individual's fitness. Mate choice in *Gryllus* spp. is an important factor affecting
340 fitness, as females will choose mates based on their various quality indicators (Scheuber et al.
341 2004). In reducing the time spent assessing this resource, females risk making error-prone
342 decisions, resulting in the choice of lesser quality males (Mowles et al. 2018). This effect of
343 anthropogenic noise on resource assessment (the resource here being the signalling male) can
344 also be seen in species using chemical and visual cues (Walsh et al. 2017). Here, lower

345 latencies could be a possible coping strategy for a reduced antipredator response. It has been
346 shown that anthropogenic noise can reduce an individual's antipredator response by
347 conflicting with signals and cues about a predator's presence (Chan et al. 2010; Kern and
348 Radford 2016). For example, great tits, *Parus major*, maximize vigilance behaviors and reduce
349 feeding behaviors during aircraft noise (Klett-Mingo et al. 2016), suggesting that predator
350 detection is reduced in these conditions. *Gryllus* spp. are also known to acoustically detect
351 predators (Miller and Surlykke 2001; Pollack and Martins 2001). By acting more quickly, the
352 females in these observations may have altered their strategy to find shelter (Hendrick and
353 Kortet 2006) or a quieter area, in order to compensate for the reduced likelihood of predator
354 detection. Interestingly, a similar, but non-significant, trend can be seen in the control
355 conditions, where a conspecific call was not present.

356 Here we have shown that the presence of anthropogenic noise affects phonotaxis
357 abilities, leading to potential consequences for an individual's reproductive success. In this
358 case, traffic noise reduced the ability of female *G. bimaculatus* to detect and approach calling
359 males using phonotaxis, compared to individuals in ambient noise conditions. Furthermore,
360 the inclusion of a white noise trial demonstrated that average amplitude alone is not enough
361 to offset phonotaxis behavior significantly, and other acoustic characteristics, such as the
362 frequency of the stimulus, fluctuations in the signal or a combination of characteristics, are
363 responsible for this conflict. The presence of traffic noise also reduced the time individuals
364 spent assessing the calling song, which could lead to selecting a lower quality mate. As similar
365 acoustic behaviors are used by a variety of taxa, these findings have important implications for
366 mate location systems throughout the animal kingdom.

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## Ethics

There are no legal requirements for studies involving orthopteran research subjects in the United Kingdom and Northern Ireland. The subject and methods of this research were approved by Anglia Ruskin University, Faculty of Science and Technology, departmental research ethics panel committee.

## Data accessibility

All data used in this experiment and analysis were uploaded to Dryad doi:10.5061/dryad.8r6p0t2

## Competing interests

We have no competing interests.

## Authors' contributions

SM & AB conceived and designed the study; AB conducted the experiments and statistical analysis with input from SM and TI. All authors wrote the manuscript.

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521

## Figure Legends

Figure 1: The number of 'correct' and 'incorrect' choices between ambient noise (n=29), traffic noise (n=28), and white noise (n=30) trials. Brackets with an asterisk show a significant result from pairwise analysis.

Figure 2: Box plot of movement latency (amount of time form start of trial to leaving the starting area) between the different acoustic conditions. Whiskers show maximum and minimum values, excluding outliers. Brackets with an asterisk show a significant results from pairwise analysis.

Electronic Supplementary material S1: Frequency spectrograms of recordings used showing **(a)** a typical *Gryllus bimaculatus* calling song, **(b)** typical traffic noise, and **(c)** computer generate white noise, with darker region showing which frequencies have the most energy at any given time. Spectrograms were created using Praat with the following properties: window length: 0.005s, time range as shown (0–5s); frequency range: 0–30000Hz.

Electronic Supplementary material S2: Audio file containing a 10 second sample of the "Song" stimulus used in this experiment.

Electronic Supplementary material S3: Audio file containing a 10 second sample of the "Traffic Noise" stimulus used in this experiment.

Electronic Supplementary material S4: Audio file containing a 10 second sample of the "White Noise" stimulus used in this experiment.

Electronic Supplementary material S5: Behavioral arena used in choice trails. Figure shows speakers embedded in both ends (black ovals) and the visible sections of the arena.

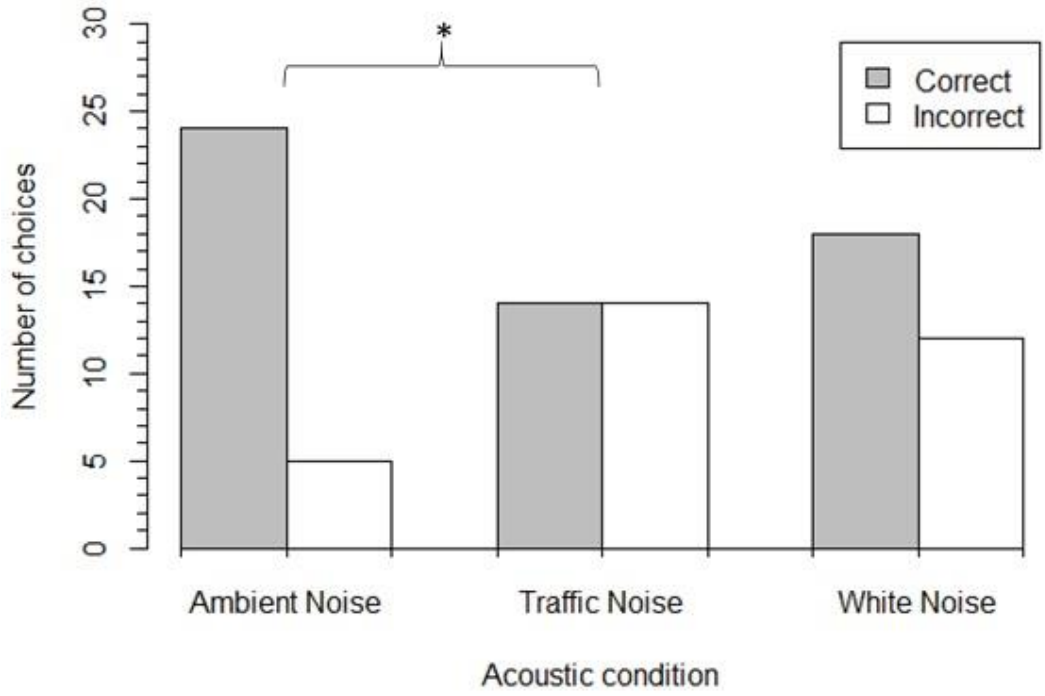
# Tables

Table 1: Output from Kruskal-Wallis tests and pairwise post hoc tests (including test statistic, sample size and p-value) on different levels of latency for standard trials and control counterparts. $\chi^2_2$ and z test statistics are reported for Kruskal-Wallis Anova and Dunn test respectively. A *n*, T *n*, and W *n* show the sample size of each noise conditions (ambient, traffic, and white, respectively) used in each test. Bold p-values indicate a significant result.

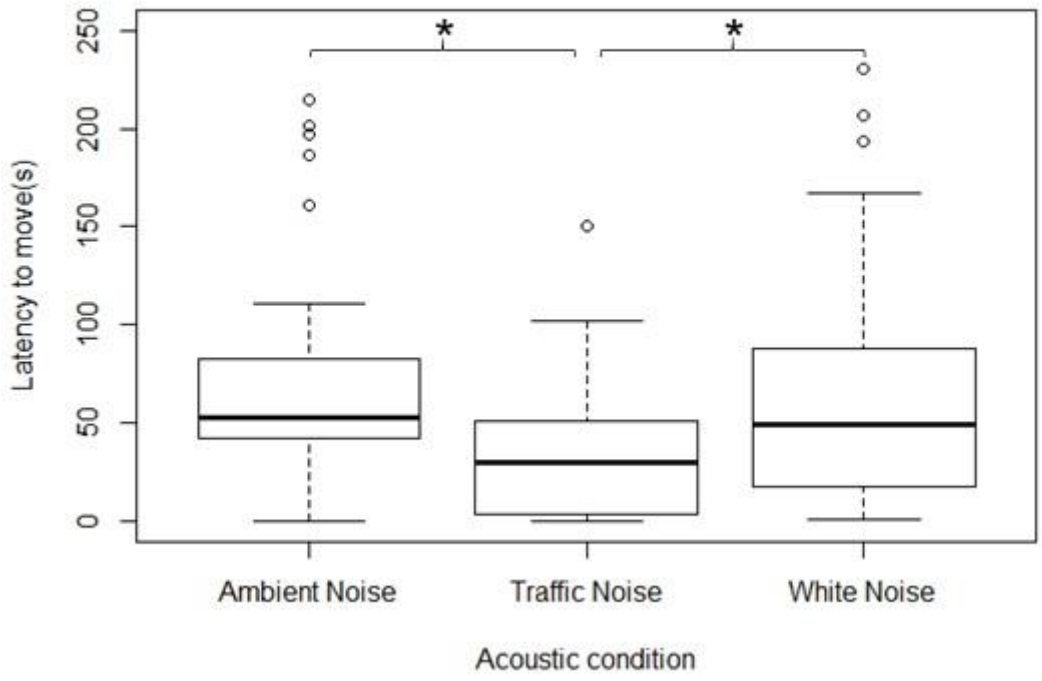
| | Statistic | A *n* | T *n* | W *n* | p | Figure |
|---|---|---|---|---|---|---|
| Latency to move | 9.672 | 29 | 28 | 30 | **0.008** | Figure 2 |
| Ambient-Traffic comparison | 3.018 | 29 | 28 | - | **0.004** | |
| Ambient-White comparison | 0.858 | 29 | - | 30 | 0.586 | |
| Traffic- White comparison | -2.192 | - | 28 | 30 | **0.043** | |
| Control | 5.787 | 28 | 28 | 26 | 0.055 | |
| Latency to choose from movement | 1.934 | 29 | 28 | 30 | 0.38 | - |
| Control | 2.854 | 28 | 28 | 26 | 0.24 | - |

# Figures

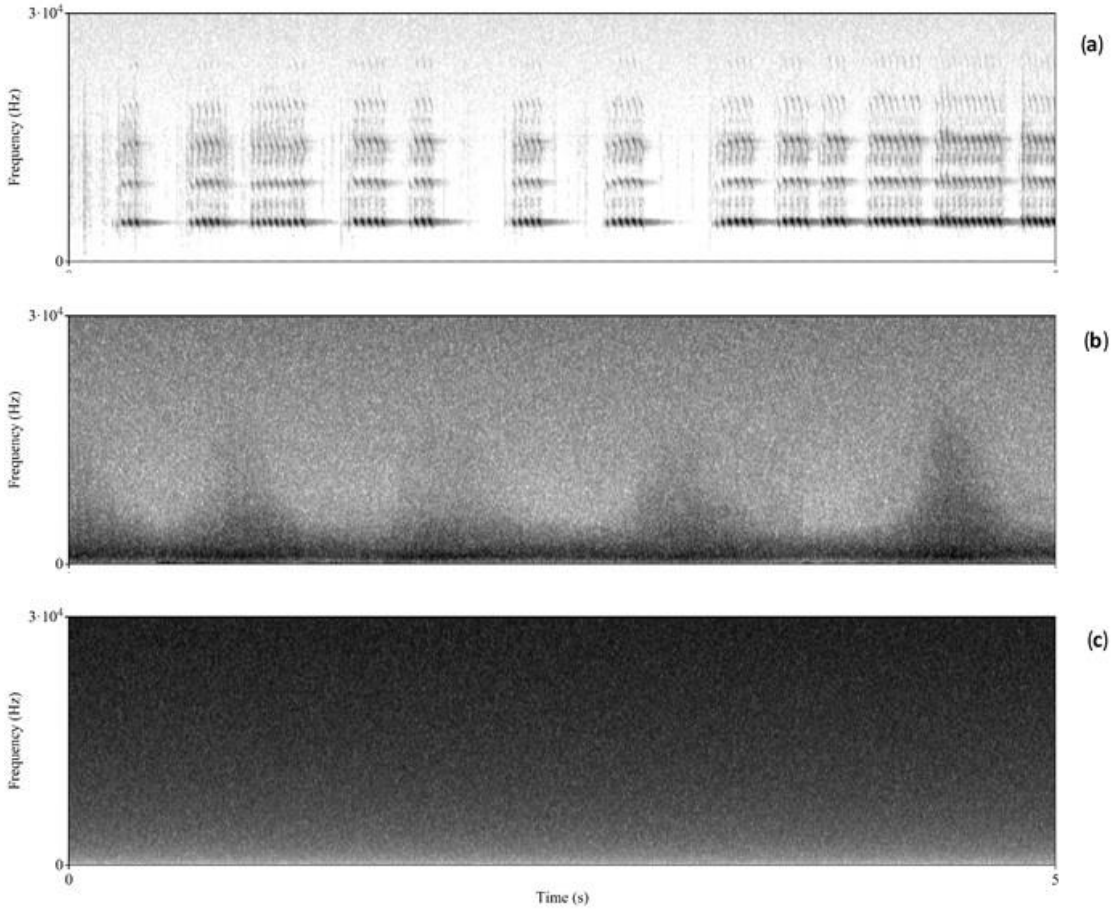
Figure 1



552 Figure 2


Latency to move(s)
0
50
100
150
200
250
*
*
Ambient Noise
Traffic Noise
White Noise
Acoustic condition

553 Supplementary Figure 1



554 Supplementary Figure 5